VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 10, Number 2, 2010

DOI: 10.1089/vbz.2008.0123

Serological Surveillance of Scrub Typhus, Murine Typhus, and Leptospirosis in Small Mammals Captured at Firing Points 10 and 60, Gyeonggi Province, Republic of Korea, 2001–2005

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Abstract

Soldiers from the Republic of Korea and the United States conducting peacetime military operations at various training sites and multiple range complexes located near the demilitarized zone separating North and South Korea are exposed to rodents and their potentially disease-carrying ectoparasites. These diseases include scrub typhus, murine typhus, and leptospirosis. Many of the training sites are rural or semi-rural, surrounded or co-located with various forms of agriculture, and are infested with rodents and insectivores (as well as their ectoparasites), which are commonly found in association with unmanaged tall grasses, scrub, and crawling vegetation habitats. For 5 years, rodents and insectivores were collected seasonally (spring, summer, fall, and winter) at firing points 10 and 60 near the demilitarized zone and serologically tested for the presence of scrub typhus, murine typhus, and leptospirosis antibodies. Of the nine species of small mammals collected, *Apodemus agrarius*, the common striped field mouse and known reservoir of scrub typhus, was the most frequently collected (90.6%). Only four of the nine species captured, *A. agrarius* (60.9%), *Micromys minutus* (100%), *Mus musculus* (55.6%), and *Rattus norvegicus* (46.7%), were positive for scrub typhus. Of all the small mammals captured, only *A. agrarius* was positive for murine typhus (0.3%) and leptospirosis (1.3%). Seasonal and annual prevalence rates based on weight and sex are presented.

Key Words: *Apodemus*—*Crocidura*—insectivores—leptospirosis—*Micromys*—*Microtus*—murine typhus—*Myodes*—*Orientia*—*Rattus*—*Rickettsia*—rodents—scrub typhus—*Tscherskia*.

Introduction

Scrub and murine typhus are vector-borne zoonotic rickettsial diseases associated with rodents and pose a major public health threat to people in many subtropical and tropical areas of the world. Similarly, they present sporadic problems among military personnel who may train or operate in field environments (Phillip et al. 1946, Yi et al. 1993, Song et al. 1996, Walker 1999, Watt and Walker 1999, Jiang et al. 2003, Jang et al. 2004, 2005, Bavaro et al. 2005, Parola and Raoult 2006, Sonthayanon et al. 2006). Chigger mites (family Trombiculidae, genus *Leptotrombidium*) vector *Orientia tsutsugamushi*, the causative agent of scrub typhus, while fleas (primarily *Xenopsylla cheopis*) vector *Rickettsia typhi*, the causative agent of murine typhus (Kawamura 1926, Zinsser 1934). *O. tsutsugamushi* is reported throughout the Korean Peninsula and Jeju Island, and has been recovered on repeated occasions throughout Korea from the common striped field

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Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE **2010**	2. REPORT TYPE	3. DATES COVERED **00-00-2010 to 00-00-2010**

4. TITLE AND SUBTITLE
Serological Surveillance of Scrub Typhus, Murine Typhus, and Leptospirosis in Small Mammals Captured at Firing Points 10 and 60, Gyeonggi Province, Republic of Korea, 2001-2005

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Naval Medical Research Center,Viral and Rickettsial Diseases Department,Silver Spring,MD,20910

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
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15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**9**	

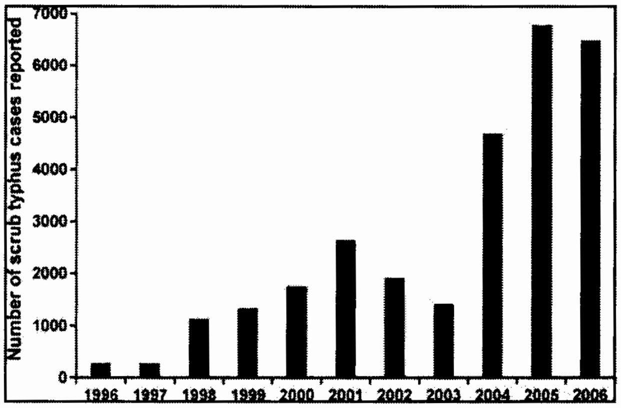

FIG. 1. Number of scrub typhus cases reported by the Korean Center for Disease Control and Prevention (KCDC), 1996–2006. Data from the KCDC (http://dis.cdc.go.kr/eng_statistics/statistics.asp) accessed on 15 May 2008.

mouse, *Apodemus agrarius* (Jackson et al. 1957, Ree et al. 1991a, 1992, 1995, Song et al. 1998). Traub and Wisseman (1974) implicated trombiculid mites, specifically *L. pallidum*, as the probable arthropod vector/host of *O. tsutsugamushi* (Munro-Faure et al. 1951, Anonymous 1953, Ley and Markelz 1961, Ree et al. 1991b). Scrub typhus was reported, but not confirmed in Korea until the first cases were identified in United Nations military personnel during the Korean War (Phillip 1949, Munro-Faure et al. 1951, Fuller and Smadel 1954, Jackson et al. 1957). After the Korean War, scrub typhus was not reported in the Korean population until the 1980s (Woo et al. 1983, Yi et al. 1986, Chang 1995). The number of cases reported increased annually and peaked at 6780 cases in 2005 (KCDC 2007) (Fig. 1). No cases have been reported among U.S. soldiers over the last decade, which may be due to the difficulty of diagnosing scrub typhus serologically during the first several weeks of infection (Kim et al. 1993, 2006, Yi et al. 1993, Jiang et al. 2003).

R. typhi was identified during the 1930s and was originally found in association with the oriental rat flea, *X. cheopis* (Barrett and Stark 1982). *R. typhi* is transmitted in flea feces that enter the bite site during feeding or are rubbed into the feeding site by the host scratching the irritated area (Gikas et al. 2004). Murine typhus, which is often characterized as an underreported mild disease with nonspecific symptoms ranging from fever and headache to nausea and vomiting, may be fatal in untreated severe cases, especially when diagnosis is delayed in older populations (Stuart and Pullen 1945, Barrett and Stark 1982). Transmission of *R. typhi* occurs horizontally, from infected flea to rodent to noninfected flea, and to a lesser degree vertically by transovarial and transstadial transmission (Azad 1990, Gikas et al. 2004). The zoonotic classic rat–flea–rat cycle present in urban environments is replaced by other vectors and zoonotic hosts in rural environments (Nogueras et al. 2006). Humans are incidental hosts, and soldiers are especially at risk of becoming infected during training exercises in disturbed rodent-infested habitats and in urban areas, especially when rodents are displaced or killed, leaving the fleas to seek blood meals from alternate hosts.

Leptospirosis, a disease caused by any one of the seven pathogenic species of *Leptospira* spirochetes, affects humans in many areas of the world and is transmitted through exposure to water or wet soil contaminated with *Leptospira*-infected urine from infected animals (Shieh et al. 1999). The importance of leptospirosis in early military history cannot be appreciated because it was not described until 1886 and isolated in 1916 (Inada et al. 1916, Yager 1954). Leptospirosis was first identified in Korea in 1984 and since has emerged as a major health problem (Lee et al. 1984). Many animal species (e.g., cattle, pigs, horses, dogs, and rodents) are hosts to the bacterium, and in many cases, the infected animals are asymptomatic. Humans infected with *Leptospira* display a broad range of usually mild symptoms that go unreported. However, a few patients develop more severe symptoms (Weil's syndrome) that may involve renal failure and hemorrhage leading to death. In addition to farming or other activities that expose persons to *Leptospira*-infected water or wet soil, outbreaks are frequently reported after periods of heavy rains and flooding (Park et al. 1990, Wuthiekanun et al. 2007). Soldiers training in and around low-land wet areas that are frequently contaminated with animal urine are at greatest risk of becoming infected with *Leptospira*.

Knowledge of the epidemiology of rodent-borne diseases is important for developing appropriate vector, reservoir, and disease mitigation strategies. In addition, an understanding of the potential disease risks associated with local rodents and their ectoparasites ensures more timely diagnosis and appropriate treatment, and allows implementation of education to minimize personnel exposure. Collectively, these ideas lead to reduced morbidity and mortality of rodent-borne and associated ectoparasite-borne diseases (Lee et al. 1981, Song et al. 1998, Ree et al. 2001, Lednicky 2003). This study reports on the serological surveillance of scrub typhus, murine typhus, and leptospirosis for rodent and insectivore populations at firing point (FP)-10 and FP-60. Subsequent papers will present rodent-borne disease surveillance, including rodent bionomics, ecology, and hantaviruses, conducted at other U.S. and Korean operated military training sites located near the demilitarized zone (DMZ).

Materials and Methods

Site description

Two Korean-operated military training sites, FP-10 and FP-60 located in Yonchon County near the DMZ, were surveyed seasonally for small mammals from the spring of 2001 through the winter season of 2005 as described by O'Guinn et al. (2008), as a result of a hantavirus case reported in October 2000 in a U.S. soldier who trained at these sites.

Sampling

Sherman folding traps (7.6×8.9×22.9 cm; H.B. Sherman, Tallahassee, FL), baited with peanut butter placed between two saltine crackers, were set along the training site perimeter as described by O'Guinn et al. (2008). Small mammals captured were transported to Korea University's biosafety level-3 laboratory, where they were anesthetized, euthanized by exsanguination after cardiac puncture to obtain a blood sample, identified to species, sexed, and weighed. Spleen, lung, and kidney samples were removed, and stored at −70°C until they were processed for diagnostic testing.

TABLE 1. NUMBER OF SMALL MAMMALS CAPTURED AT FP-10 AND FP-60 THAT WERE SEROPOSITIVE FOR SCRUB TYPHUS, MAY 2001 THROUGH DECEMBER 2005

Species	*FP-10*	*FP-60*	*Total*
Apodemus agrarius	358/520 (68.8%)[a]	353/647 (54.6%)	711/1167 (60.9%)
Micromys minutus	1/1 (100.0%)	4/4 (100.0%)	5/5 (100.0%)
Mus musculus	3/6 (50.0%)	2/3 (66.7%)	5/9 (55.6%)
Rattus norvegicus	2/4 (50.0%)	5/11 (45.5%)	7/15 (46.7%)
Rattus rattus	0	0/1 (0.0%)	0/1 (0.0%)
Tscherskia triton	0	0/6 (0.0%)	0/6 (0.0%)
Microtus fortis	0/1 (0.0%)	0/2 (0.0%)	0/3 (0.0%)
Myodes regulus	0/1 (0.0%)	0/1 (0.0%)	0/2 (0.0%)
Crosidura lasiura	0/24 (0.0%)	0/56 (0.0%)	0/80 (0.0%)
Total	364/557 (65.4%)	364/731 (49.8%)	728/1288 (56.5%)

[a]Number seropositive/number tested (percentage seropositive).
FP, firing point.

Data collection

An electronic summary data sheet was prepared that included a unique specimen identification number, species, sex, weight (g), gravid state of females (2003–2005), and antibody status for hantaviruses (JW Song, personal communication), scrub typhus, leptospirosis, and murine typhus.

Indirect immunofluorescence assay for scrub typhus and murine typhus

Rodent sera were diluted 1:32 in phosphate-buffered saline (PBS), and the total amount of immunoglobulin G against *O. tsutsugamushi* (Karp and Gilliam strains) and *R. typhi* was determined by an indirect immunofluorescence assay. Antigen-coated slides were derived from yolk sac culture preparations (Gimenez 1964, Robinson et al. 1976). Diluted serum (30 μL) starting at a 1:32 dilution was pipetted onto the antigen-spotted slide and incubated at 37°C for 30 min in a humidified chamber. The slides were washed three times for 3 min each with PBS (10 mM, pH 7.2). Fluorescein isothiocyanate–conjugated goat anti-mouse or anti-rat antibody (30 μL; MP Biomedicals, Aurora, OH), was pipetted onto each spot, and the slides were then incubated in a humidified chamber at 37°C for 30 min. The slides were washed as described above and then air-dried. The buffered glycerin-mounted slides were then examined for specific fluorescence using a fluorescence microscope (50 W; Zeiss, Mainz, Germany). Appropriate positive and negative sera were used as controls, and previous studies indicate that the detection antibodies used in this study would detect each of the rodent/insectivore sera tested (Ree et al. 1991a, 1995, Yamashita et al. 1994, Siritantikorn et al. 2003, JWS, unpublished data).

Macroagglutination test for leptospirosis

Macroagglutination tests were conducted only on small mammals that arrived live at the laboratory. *Leptospira* antigen was prepared by inoculating EMJH (Ellinghausen and McCullough as modified by Johnson and Harris) medium (Difco Laboratories, Sparks, MD) with *Leptospira interrogans* serovar Lai followed by incubation in a shaking incubator at 30°C for 5–6 days. The spirochete culture was clarified by centrifugation at 750 *g* for 10 min. The spirochetes were then partially purified from the supernatant by centrifugation at 12,000 *g* for 30 min. The spirochete pellet was combined with 10 mL of Galton solution (0.5% formalin, 12% NaCl, and 20% glycerin)/250 mL of EMJH medium and mixed using a 16–18-gauge needle and volumetric syringe. The antigen was then stored for 4 days at 4°C, clarified by centrifugation at 750 *g* for 5 min, and the resulting supernatant adjusted to an optical density of 41 at 550 nm with Galton solution. The macroagglutination test for leptospirosis of rodent serum was performed at a 1:4 dilution (8 μL of serum mixed with 24 μL of bacterial antigen solution). This was then spotted on a ring slide and incubated at room temperature in an orbital shaker at 125 rpm for 4 min. Serum was determined positive for anti-*Leptospira* antibodies if fine to coarse clumps were observed in a dark background under the microscope.

Statistics

Data were maintained in an Excel file (Microsoft, Seattle, WA) and analyzed descriptively. Comparative analysis was performed using SPSS (SPSS, Chicago, IL) using cross-the χ^2 test; with α set at 0.01.

Animal use

The collection, handling, and euthanasia of small mammals were conducted under animal use guidelines, College of Medicine, Korea University.

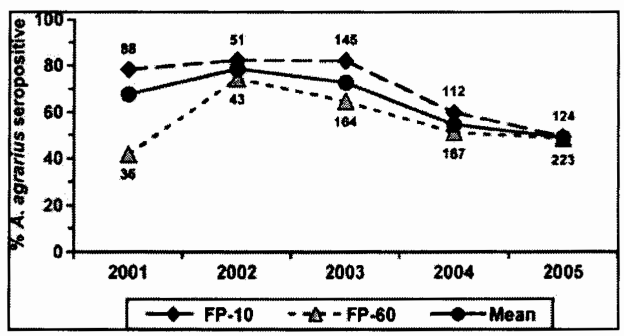

FIG. 2. Mean annual percent of *Apodemus agrarius* seropositive for scrub typhus at firing point (FP)-10 and FP-60, 2001–2005.

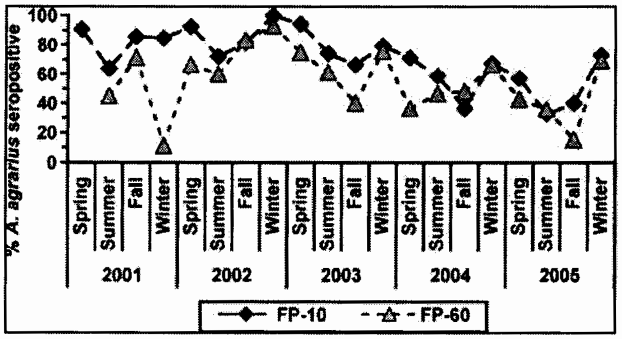

FIG. 3. Seasonal percentage of *Apodemus agrarius* seropositive for scrub typhus at FP-10 and FP-60, 2001–2005.

Results

Scrub typhus

Only four species of rodents (*A. agrarius*, *Micromys minutus*, *Rattus norvegicus*, and *Mus musculus*) were serologically positive for scrub typhus at FP-10 and FP-60, while none of the *Crocidura lasiura* (insectivore) ($n = 80$), *Microtus fortis* ($n = 3$), *Tscherskia* (=*Cricetulus*) *triton* ($n = 6$), *Myodes* (=*Eothenomys*) *regulus* ($n = 2$), or *R. rattus* ($n = 1$) were seropositive (Table 1). While only five *M. minutus* were captured, all (100%) were seropositive for scrub typhus; this was followed by *A. agrarius* (60.9%), *M. musculus* (55.6%), and *R. norvegicus* (46.7%). Annual seropositive rates for *A. agrarius* were similar at both FPs, with mean annual seropositive rates ranging from 48.8% to 78.7% (Fig. 2), except for 2001, when seropositive rates at FP-60 were approximately one half of that observed at FP-10. Overall, seasonal seropositive rates were higher at FP-10 than at FP-60 (Fig. 3). Significantly fewer *A. agrarius* were seropositive for scrub typhus during the summer (51.0%) and fall (48.0%) trapping periods than during the winter (71.2%) and spring (71.9%) periods ($\chi^2 \geq 24.7$, df = 1, $p < 0.001$). However, these rates were not synchronous; that is, during some periods, rates were high at one training site while low at the other site and ranged from a low of 11.1% to a high of 100% (Table 2; Fig. 3). The numbers of other captured/seropositive rodent species were too low to determine seropositive rate trends (Table 2).

Scrub typhus seropositive rates among male *A. agrarius* were similar at FP-10 (50.3%) and FP-60 (53.0%) and were not significantly different from seropositive rates observed in females ($\chi^2 = 0.59$, df = 1, $p < 0.44$). Additionally, overall annual and seasonal seropositive rates for *A. agrarius* males and females for both FP-10 and FP-60 were similar.

Scrub typhus seropositive rates were generally higher for all weight classes, especially for those weighing between 30 and 40 g, at FP-10 than at FP-60, which were 84.2% and 53.9%, respectively (Fig. 4). During the spring and winter, scrub typhus seropositive rates for *A. agrarius* were similar for all weight classes, whereas during the summer, there was a significant increase in the seropositive rates of older (i.e., >30 g) than in younger (i.e., <30 g) populations ($\chi^2 = 39.8$, df = 1, $p < 0.001$) (Table 3; Fig. 5).

Murine typhus

Only 0.4% (2/520) and 0.3% (2/647) of *A. agrarius* were positive for murine typhus, an urban flea-borne disease, at FP-10 and FP-60, respectively (Table 4). All other rodent and insectivore species ($n = 121$) were negative for murine typhus.

Leptospirosis

Leptospirosis was infrequently observed at both FP-10 and FP-60. Of the 509 small mammals that were tested for leptospirosis, only *A. agrarius* were seropositive (1/220 [0.5%] from FP-10 and 5/237 [2.3%] from FP-60) (Table 5). The remaining 52 small mammals belonging to the other eight genera of small mammals were seronegative for leptospirosis.

Discussion

A comprehensive rodent-borne disease surveillance program that included serosurveillance of hantaviruses, scrub typhus, murine typhus, and leptospirosis was initiated at FP-10 and FP-60 as a result of a U.S. soldier acquiring hemorrhagic fever with renal syndrome attributed to exposure at one of these sites while conducting field training during September–October of 2000. A serosurvey of scrub typhus in small mammal populations was included due to the number of reported cases among the Korean populations that increased from 1758 in 2000 to nearly 7000 cases by 2005. These increases, while not well understood as overall seropositive

TABLE 2. NUMBER OF SMALL MAMMALS CAPTURED AT FP-10 AND FP-60 SEROPOSITIVE FOR SCRUB TYPHUS BY SEASON, MAY 2001 THROUGH DECEMBER 2005

Species	Trapping period (FP-10 and FP-60)				Total
	Spring	Summer	Fall	Winter	
Apodemus agrarius	187/260 (71.9%)	203/398 (51.0%)[a]	86/179 (48.0%)	235/330 (71.2%)	711/1167 (60.9%)
Micromys minutus	3/3 (100.0%)	2/2 (100.0%)	0	0	5/5 (100.0%)
Mus musculus	1/3 (33.3%)	3/5 (60.0%)	0	1/1 (100.0%)	5/9 (55.6%)
Rattus norvegicus	1/1 (100.0%)	5/7 (71.4%)	1/7 (14.3%)	0	7/15 (46.7%)
Rattus rattus	0	0	0/1 (0.0%)	0	0/1 (0.0%)
Tscherskia triton	0/1 (0.0%)	0/5 (0.0%)	0	0	0/6 (0.0%)
Microtus fortis	0	0/1 (0.0%)	0/2 (0.0%)	0	0/3 (0.0%)
Myodes regulus	0/1 (0.0%)	0	0	0/1 (0.0%)	0/2 (0.0%)
Crosidura lasiura	0/10 (0.0%)	0/3 (0.0%)	0/13 (0.0%)	0/54 (0.0%)	0/80 (0.0%)
Total	192/279 (68.8%)	213/421 (50.6%)	87/202 (43.1%)	236/386 (61.1%)	728/1288 (56.5%)

[a]Number of animals seropositive for scrub typhus/total number of animals tested (percentage seropositive).

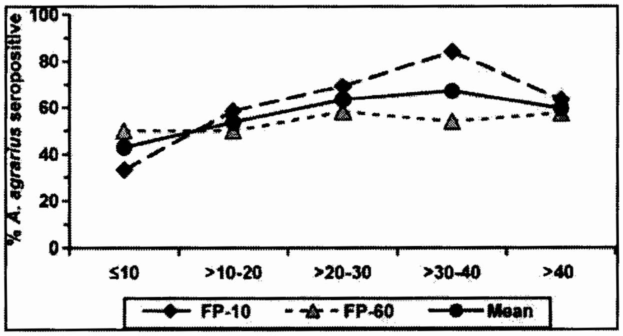

FIG. 4. Overall percentage of (mean) *Apodemus agrarius* seropositive for scrub typhus, by weight, at FP-10 and FP-60, 2001–2005.

rates among *A. agrarius* (primary reservoir) populations sampled at the survey sites declined from a high of 78.7% in 2002 to a low of 48.8% in 2005, may be due, in part, to an improved reporting system and a public law established in August 2000 prohibiting the dispensing of antibiotics without a doctor's prescription. While no cases of scrub typhus have been reported in U.S. soldiers over the last decade, serological techniques used for their diagnosis are often not positive for up to 30 days after infection, and follow-up characterization of the illness is often not done once the soldier is released to return to duty (Jiang et al. 2003).

Overall observed prevalence rates for scrub typhus ranged from 32.6% to 100% and from 11.1% to 92.9% at FP-10 and FP-60, respectively, and were similar to survey results of Ree et al. (1995, 1997b, 2001), who found prevalence rates ranged 0.0–81.1% at various localities throughout Korea. In another study, Ree et al. (1991a) reported overall prevalence rates of 41.8%, which was lower than the observed mean prevalence rates at FP-10 (68.8%) and FP-60 (54.6%). Jackson et al. (1957), Shim et al. (1989), and Kim et al. (1990) also reported much lower overall prevalence rates of 14.3%, 28.8%, and 22.4%, respectively. These lower rates may be a result of types of habitats surveyed, various localities sampled, trapping period (lower rates observed during the summer and fall), and low numbers of small mammals sampled. Similar to Ree (1991a), we reported scrub typhus infections in *R. norvegicus*, *M. minutus*, and *A. agrarius*. Ree (1991a) also reported infections in *R. rattus* and *M. fortis*, but our collection numbers for these species were too low for analysis. All 80 *C. lasiura* collected from grassy habitats where infected *A. agrarius* were commonly found were negative for scrub typhus, which was similar to the results of others (Jackson 1957, Woo et al. 1983, Shim et al. 1989, Kim et al. 1990, Lee et al. 1990, Ree et al. 1991a). However, 3/117 (2.6%) *C. lasiura* at other sites near the DMZ and in another study 1/8 (12.5%) were positive for scrub typhus antibodies (Song et al. 1998, Klein, unpublished data). The low numbers of *C. lasiura* seropositive for scrub typhus may be due to the predominance of nonvector chiggers (*Neotrombicula tamiyai*, mean 9.3/insectivore) infesting this insectivore and low infestation rates of vector chiggers (*L. orientale*, mean 0.3/insectivore) in this and other studies (Ree et al. 1997a, Kim personal communication).

Overall, seropositive rates were significantly higher at FP-10 than at FP-60 ($\chi^2 = 24.1$, df = 1, $p < 0.001$), and seasonal seropositive rates were consistently higher at FP-10 than at FP-60. The lower rates at FP-60 may be due to habitat differences, as one trap line and a portion of another trap line consisted mostly of nongrassy habitats (broadly spaced herbaceous plants) that may not have been conducive to large populations of chiggers. Similar to Ree et al. (1991a), the prevalence of scrub typhus antibodies was highest during the winter and spring trapping periods (>70%) and lowest during the summer and early fall trapping periods ($\sim$50%). These lower rates during the summer may be a result of higher reproductive rates (gravid females) during the early summer (4.2% gravid females) and a younger population. However, when the reproductive rates were higher during the fall (27.2% of captured females were gravid) (O'Guinn et al. 2008), the prevalence rate increased the following winter. Thus, these differences in prevalence rates cannot be explained solely by the infusion of young rodents into the general population. Additionally, prevalence rates of younger *A. agrarius* (<20 g) were similar to those of older populations (>30 g).

Scrub typhus is maintained exclusively through transovarial transmission from the adult nonparasitic mite to the parasitic larvae of the next generation and typically occurs in isolated and sharply defined areas (Mackie et al. 1946, Audy 1949). According to Traub and Wisseman (1974), epidemics of scrub typhus depend on four factors: (1) the presence of *Leptotrombidium* mites; (2) the presence of *O. tsutsugamushi*; (3) a high incidence of infected wild rodents; and (4) the occurrence of transitory vegetation in a disturbed environment; all of which are characteristic of military training sites located near the heavily fortified DMZ that separates North and South Korea along the 38th parallel. Scrub typhus often occurs in nature in small, but intense, foci when an appropriate combination of parasites, vectors, and hosts come together (Ree et al. 1991b, Benenson 1995, Varma 1996). These same factors apply to other zoonotic diseases involving small mammals as reservoir hosts—for example, hantavirus disease

TABLE 3. THE NUMBER OF *APODEMUS AGRARIUS* SEROLOGICALLY POSITIVE FOR SCRUB TYPHUS/NUMBER CAPTURED (PERCENT INFECTED) AT FP-10 AND FP-60, BY WEIGHT, MAY 2001 THROUGH DECEMBER 2005

			Weight (g)			
Trapping seasons	*≤10*	*>10–20*	*>20–30*	*>30–40*	*>40*	*Total*
Spring	1/2 (50.0)[a]	29/49 (59.2)	91/124 (73.4)	60/78 (76.9)	6/7 (85.7)	187/260 (71.9)
Summer	1/5 (20.0)	23/84 (27.4)	81/176 (46.0)	69/98 (70.4)	29/35 (82.9)	203/398 (51.0)
Fall	4/6 (66.7)	11/16 (68.8)	7/18 (38.9)	39/75 (52.0)	25/64 (39.1)	86/179 (48.0)
Winter	0/1 (0.0)	117/186 (62.9)	95/112 (84.8)	16/23 (69.6)	8/8 (100.0)	236330 (71.5)
Total	6/14 (42.9)	180/335 (53.7)	274/430 (63.7)	184/274 (67.2)	68/114 (59.6)	712/1167 (61.0)

[a]Number seropositive/number tested (percent seropositive).

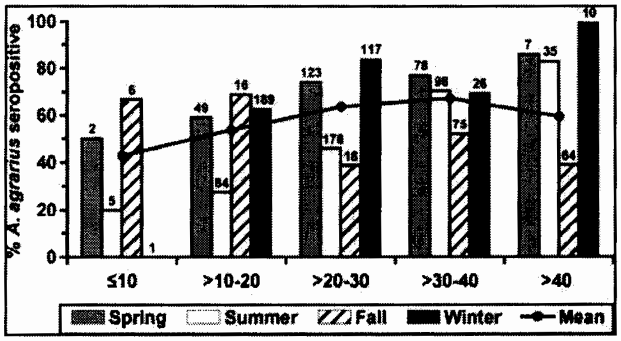

FIG. 5. Seasonal percentage of (mean) *Apodemus agrarius* seropositive for scrub typhus, by weight, at FP-10 and FP-60, 2001–2005.

TABLE 5. SMALL MAMMALS POSITIVE FOR LEPTOSPIROSIS AT FP-10 AND FP-60, 2001–2005

Species	*FP-10*	*FP-60*	*Total*
Apodemus agrarius	1/220 (0.5%)[a]	5/237 (2.1%)	6/457 (1.3%)
Micromys minutus	0	0/2 (0.0%)	0/2 (0.0%)
Mus musculus	0/1 (0.0%)	0	0/1 (0.0%)
Rattus norvegicus	0/3 (0.0%)	0/9 (0.0%)	0/12 (0.0%)
Tscherskia triton	0	0/2 (0.0%)	0/2 (0.0%)
Microtus fortis	0/1 (0.0%)	0/2 (0.0%)	0/3 (0.0%)
Myodes regulus	0	0/1 (0.0%)	0/2 (0.0%)
Crosidura lasiura	0/12 (0.0%)	0/19 (0.0%)	0/31 (0.0%)
Total	1/237 (0.4%)	5/272 (1.8%)	6/509 (1.2%)

[a]Number seropositive/number tested (percentage seropositive).

and tick-borne diseases. Humans often become targets when their activities bring them in contact with ectoparasites (e.g., fleas, ticks and mites) seeking alternate hosts due to a decrease in available natural host populations. This situation allows for the transmission by the arthropod vectors of the agents of scrub typhus, murine typhus, ehrlichiosis, spotted fever group rickettsioses, tick-borne encephalitis, and other diseases to humans.

The proximity of cantonment sites (often abutting to tree lines and grassy rodent habitats for camouflage) and the concentration of refuse left overnight may attract rodents and other animals, thereby increasing the potential for rodent/insectivore contact (and the ectoparasites that they harbor) with human hosts. This situation increases risk for soldier-contracted arthropod-borne diseases and is especially true for scrub typhus transmission. In addition, soldiers who are deployed to and train in Korea are often not aware that they have been bitten by chigger mites, because, unlike bites from many of the North and South American chiggers (which are intensely itchy), bites by chiggers found in Korea are often painless and produce little skin irritation (Goddard 2000). Thus, if soldiers become ill with scrub typhus, they do not inform the medical provider that they received bites from chiggers, making the diagnosis much more problematic.

Leptospira spp., the causative agent of leptospirosis, are spiral-shaped bacteria that occur worldwide with the highest

TABLE 4. SMALL MAMMALS SEROPOSITIVE FOR MURINE TYPHUS AT FP-10 AND FP-60, 2001–2005

Species	*FP-10*	*FP-60*	*Total*
Apodemus agrarius	2/520 (0.4%)[a]	1/647 (0.2%)	3/1167 (0.3%)
Micromys minutus	0/1 (0.0%)	0/4 (0.0%)	0/5 (0.0%)
Mus musculus	0/6 (0.0%)	0/3 (0.0%)	0/9 (0.0%)
Rattus norvegicus	0/4 (0.0%)	0/11 (0.0%)	0/15 (0.0%)
Rattus rattus	0	0/1 (0.0%)	0/1 (0.0%)
Tscherskia triton	0	0/6 (0.0%)	0/6 (0.0%)
Microtus fortis	0/1 (0.0%)	0/2 (0.0%)	0/3 (0.0%)
Myodes regulus	0/1 (0.0%)	0/1 (0.0%)	0/2 (0.0%)
Crosidura lasiura	0/24 (0.0%)	0/56 (0.0%)	0/80 (0.0%)
Total	2/557 (0.4)	1/731 (0.1%)	3/1288 (0.2%)

[a]Number seropositive/number tested (percentage seropositive).

incidences in tropical and subtropical regions and consists of >200 serovars that are specific for animal hosts (Farr 1995, Levett 2001). *Leptospira* parasites are shed in the urine of host animals and contracted when the bacteria enter through abrasions of the skin when animals, including humans, enter water sources, for example, slow-moving streams/rivers, ponds, ditches, sewers, and swimming pools. Rodents are the natural host of *L. interrogans icterohemorrhagiae* and serological evidence demonstrates a widespread occurrence in Korea (Cho 1989, Chang et al. 1993, Cho et al. 1998, 1999). Takaki (1919) first reported *L. interrogans icterohemorrhagiae* from a skunk, and in 1984 the first human cases of leptospirosis were identified in Korea (Lee et al. 1984, Cho et al. 1998). Since then, leptospirosis has emerged as one of the major public health threats with the Korean Center for Disease Control and Prevention (KCDC) reporting a range of 83–133 cases among the Korean population from 2001 to 2005 (KCDC 2007). Infections are often unreported because they are relatively mild, ranging from asymptomatic to flu-like symptoms. However, a more serious form (Weil's disease) may be fatal. Overall, previous reports of leptospirosis in rodent surveys ranged from 3.2% to 18.2% and demonstrate a wide distribution and prevalence (Cho et al. 1998). Human outbreaks among rice farmers were often associated with flooding rice fields before harvesting (Park et al. 1989, 1990). A report by Chang et al. (1993) showed that >10% of all febrile patients from four laboratories were positive for leptospirosis, especially in the autumn, with estimates from this and other studies suggesting that the actual number of cases is approximately five times higher than the reported number. While most cases resolve naturally without treatment, the mortality rate among severe cases may exceed 20% in untreated patients or patients who receive untimely or inappropriate treatment (Daher et al. 1999). Under reporting of cases among Korean populations is attributed to a widely used polyvalent vaccine and relatively mild symptoms in a majority of infections where patients do not seek medical care and go untreated and undocumented (Cho et al. 1998, Levett 1999, KCDC 2007).

Murine typhus is often described as an urban disease transmitted by the oriental rat flea. The prevalence rates at FP-10, FP-60, and at seven other training sites (Chaparral Range, Local Training Area 130, Warrior Base, Rodriguez Range, North Carolina Range, Monkey 7, and Dagmar North) near the DMZ (unpublished data) were very low. These sites are not near villages or dense populations of human habitat, which may impact and increase the potential for this disease.

Risk assessment for disease and nonbattle injuries among U.S. soldiers training in field environments is essential to reduce morbidity and mortality due to often preventable diseases. While there have been no cases of scrub typhus among U.S. soldiers documented in the past 10 years attributed to exposure in Korea, postdeployment screening for antibodies to rickettsial pathogens suggest an infection rate exceeding 3% (Richards, unpublished data). As discussed above, the failure to diagnose scrub typhus in U.S. soldiers may be due to relatively mild symptoms in a majority of cases and the difficulty in detecting an antibody response that occurs 5–10 days after the initial symptoms of disease. In Korea, one of the authors (T.A.K.) observed several soldiers with typical symptoms, including eschars, but all were serologically negative, perhaps due to the delayed antibody response. This was recently the case among U.S. Marines training at Camp Fuji, Japan (Jiang et al. 2003). Knowing scrub typhus poses a potential serious health threat and that it can incapacitate whole units (Southeast Asia during World War II), provides commanders with the impetus to implement preventive medicine measures, for example, proper wear (trouser legs tucked into the boots) and impregnation of the uniform with permethrin to kill/repel larval chigger mites (Mackie et al. 1946, Fuller and Smadel 1954, Ley and Markelz 1961). Additionally, in some areas, grasses should be cut short, rodent populations controlled, and pesticides applied to reduce vector populations. For example, rodent populations were very low at sites that were trapped where the grass was maintained at <10 cm in height (e.g., Air Force installations and some rice paddy banks), and maintaining environments that increase rodent predation where soldiers actively train may mitigate risks of disease transmission (Burkett 2001). These strategies need to be further evaluated to determine the effectiveness of preventive medicine measures, vector, and rodent control through analysis of pre- and postdeployment blood samples to determine if transmission rates decrease after the implementation of these policies—policies that worked well in WWII.

Acknowledgments

We acknowledge the assistance of Wan-Young Kim, Edwin Huertas, and Kerry McKinley for their support in coordinating personnel for conducing rodent surveillance. We also thank many soldiers of the 5th, 38th, and 154th Medical Detachments (Preventive Medicine) and 168th Multifunctional Medical Battalion, for their support in conducting rodent surveillance throughout the 5-year surveillance program. We especially thank the graduate students and staff of Korea University for their support for bionomic data collection for the small mammals. We acknowledge Jong-Koo Lee, Director General, KCDC, and his staff for their administrative support and reports on scrub typhus in Korean populations. We are grateful for the support and encouragement that the 18th MEDCOM commanders, Phillip Volpe and Brian Allgood (deceased), and their preventive medicine consultant Hee-Choon Lee provided throughout this study.

Funding for portions of this work was provided by the U.S. Department of Defense Global Emerging Infections Surveillance and Response System, Silver Spring, MD, and the Armed Forces Medical Intelligence Center, Fort Detrick, MD.

Disclosure Statement

The opinions, interpretations, conclusions, and recommendations contained herein are those of the authors and do not reflect official views or policy of the U.S. Army, the U.S. Department of Defense, or the Ministry of Health, Republic of Korea. No competing financial interests exist.

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